84-2 Salmonella Mutagenicity Test
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DATA EVALUATION REPORT

Study Type: Gene Mutation in Bacteria — **TOX. CHEM. NO.:** 298C

Accession No.: 403888-05 — **MRID NO.:**

Test Material: CGA-154281 Technical (Batch No. FL870116; 99% Purity)

Synonyms:

Study Number (s): 871077

Sponsor: CIBA-GEIGY Corporation Agricultural Division

Testing Facility: CIBA-GEIGY Limited Experimental Pathology Laboratory, Basal, Switzerland

Title of Report: Salmonella/Mammalian-Microsome Mutagenicity Test (Test material: CGA 154281 technical)

Author(s): Dr. B. Ogorek and Prof. D. Muller

Report Issued: June 30, 1987

Conclusions:

CGA-154281 technical was nonmutagenic to TA98, TA100, TA1535 and TA1537 strains of Salmonella typhimurium either with or without metabolic activation at the concentrations tested.

Concentrations tested: 20, 78, 313, 1250 and 5000 ug/plate

Classification of Data: Unacceptable
(Deficiencies are identified in the detailed review)

Title of Study: Salmonella/Mammalian-Microsome Mutagenicity Test with CGA-154281 Technical
Ciba-Geigy Limited Experimental Pathology Test No. 871077

I. Materials and Methods:

1. Test Materials:

The test compound, CGA-154281 technical (Batch No. FL870116; 99% Purity), was dissolved in acetone. Solutions of daunorubicin-HCl in phosphate buffer, 4-nitroquinoline-N-oxide in phosphate buffer, N-methyl-N'-nitro-N-nitroso-guanidine in phosphate buffer, 9-aminoacridine hydrochloride in DMSO, cyclo-phosphamide in phosphate buffer and 2-aminoanthracene in DMSO were prepared prior to use and served as positive controls.

2. Bacteria

Four histidine-auxotrophic strains of Salmonella typhimurium (TA98, TA100, TA1535 and TA1537) originally obtained from Dr. Ames were used in this study.

3. In Vitro Metabolic Activation System

The mammalian metabolic activation system consisted of rat liver homogenate from Aroclor 1254-treated rats (Tif:RAIF(SPF)) and a solution of co-factors described by Ames et al. (Mutation Res., 31: 347-364, 1975).

4. Mutagenicity Test

The mutagenicity tests were carried out in accordance with the method described by Ames et al. (1975). The mutagenicity of CGA-154281 technical was evaluated by the Ames test at the concentrations of 20, 78, 313, 1250 and 5000 ug/plate either in the presence or absence of metabolic activation. Mutations were quantified on triplicate plates for each strain by counting the his^+ revertant colonies after 48 hours of incubation at 37°C on a selective agar plate. Positive control compounds and negative (solvent) control were run concurrently with the test compound.

5. Evaluation Criteria

The test compound is considered to be positive in this test system if one of the following conditions are met:

(A) A reproducible doubling of the mean number of revertants per plate above that of the negative control at any concentration level for one or more of the following strains: TA98, TA1535 and TA1537;
(B) A reproducible increase of the mean number of revertants per plate for any concentration above that of the negative control by a factor of 1.5 for strain TA100;
(C) Dose-response relationship

II. Reported Results: (Tables 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 attached)

1. Primary Toxicity Test

Nine concentrations of CGA-154281 technical (i.e., 0.08, 0.31, 1.2, 4.9, 19.5, 78.1, 312.5, 1250 and 5000 ug/plate) were tested to determine the highest concentration to be used in the mutagenicity assay. From the results obtained (Tables 1 and 6), a reduction in the revertant colonies was observed in the CGA-154281-treated cultures of TA100 and TA1537 strains at the concentration of 5000 ug/plate. Also, at the concentrations of 1250 and 5000 ug/plate, the material precipitated in soft agar. Therefore, the highest concentration suitable for the mutagenicity test was found to be 5000 ug/plate.

2. Mutagenicity Test

No increase in the number of revertant colonies (less than 2-fold) over concurrent control value was observed for any of tester strains following exposure to the test compound (i.e., 20, 78, 313, 1250 and 5000 ug/plate) either in the presence or absence of metabolic activation.

III. Evaluation and Recommendation:

1. The specific procedures used for confirming the genotypes of TA98, TA100, TA1535, and TA1537 strains of Salmonella typhimurium in accordance with the individual sensitivity test recommended by the Ames test were not given in the report.

2. The spontaneous revertant colonies for each of the four tester strains of Salmonella typhimurium are found within the normal ranges of revertant colonies recommended by the Ames test (Mutation Res., 31: 347-364, 1975).

3. The strain specific control compounds (daunorubicin-HCl; 4-nitroquinoline-N-oxide; N-methyl-N'-nitro-N-nitrosoguanidine; 9-aminoacridine-HCl) and the positive controls (cyclophosphamide; 2-aminoanthracene) to ensure the efficacy of the activation system have given the strong positive responses as expected.

4. Since the cytotoxicity of the test compound against TA100 and TA1537 strains was observed at 5000 ug/plate in primary toxicity test (See results in Tables 1 and 6), the highest dose of CGA-154281 technical (5000 ug/plate) used in this study is considered acceptable. However, the assay should be evaluated using a minimum of five concentrations with adequate intervals between test points (i.e., a narrow range of concentrations recommended). It is, therefore, questionable whether appropriate median doses of the test compound (i.e., 2000, 3000 or 4000 ug/plate) were chosen for this study. The study is judged unacceptable in the present form.

4-(Dichloroacetyl)-3,4-Dihydro-3-Methyl-
2H-1,4-Benzoxazine

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